

## Genetics of Type I Diabetes Mellitus: A Single, Recessive Predisposition Gene Mapping between *HLA-B* and *GLO*

With an Appendix on the Estimation of Selection Bias by C. Falk

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### SUMMARY

Three different published sets of HLA-typed families of juvenile diabetes mellitus (JDM) patients have been analyzed. There was no significant genetic heterogeneity between them according to the criterion of Morton, and the total material was analyzed on the assumption of a single recessive (*JDM-P*) gene with incomplete penetrance. The analysis, carried out with the NYLIP program modified to account for penetrance  $< 1$  and for selection bias, yields highly significant lod scores for linkage between *HLA* and *JDM-P*, with a maximum value of 7.40 at  $\theta = .05 \pm .03$ .

The segregation of *HLA* and *GLO* in five affected sib pairs, in which one of the sibs carries an *HLA/GLO* recombinant, places *JDM-P* closer to *HLA* than the *GLO* locus: four of these five pairs are *HLA*-identical and *GLO*-different, in agreement with the conclusions of the formal linkage analysis.

The data from these three independent sets of families are therefore consistent with our earlier claim that JDM is inherited as a recessive trait closely linked to *HLA* with reduced penetrance, and its analysis does not require more complicated genetic models.

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## INTRODUCTION

The identification of *HLA* as a genetic marker for type I (insulin-dependent or juvenile) diabetes mellitus, hereafter called JDM, is based on the demonstration of linkage between *HLA* and the genetic predisposition to this type of diabetes.

Shortly after Singal and Blajchman [1] and Nerup et al. [2] demonstrated the population associations between *HLA* and JDM, Cudworth and Woodrow [3] showed that affected sib pairs shared *HLA* haplotypes much more often than would be predicted on the assumption of genetic independence of these traits. Using families in which all members were *HLA*-typed, we confirmed the excess of haplotype sharing by affected sib pairs [4]. We then suggested [5] recessivity of the disease because the affected sib pairs shared both *HLA* haplotypes significantly more frequently than would be expected from the Mendelian segregation of the identified parental haplotypes. These family studies were subsequently extended permitting us to estimate the penetrance to the proposed *JDM* homozygous siblings of patients (i.e., those sharing with the propositi both *HLA* haplotypes) to be similar in magnitude to the concordance rate for monozygotic twins of patients [6]. Using this approximation to the penetrance under the assumption of recessivity of a susceptibility gene, we then calculated the lod score for linkage between *HLA* and *JDM* and reported the results of the formal genetic analysis of 37 families at the 4th International Workshop on Human Gene Mapping in July 1977 [7]. The maximum lod score was 4.89 at a recombination frequency of .04. Intra-*HLA* recombinants in these families enabled us to locate the *JDM* predisposition gene (*JDM-P*) near the *D* end rather than the *A* end of *HLA* [4, 5, 7]. Using an elegant sib-pair analysis method, applicable to incompletely typed families, Thomson and Bodmer [8] reanalyzed Cudworth and Woodrow's affected sib-pair data [3] and found that the data also best fit the hypothesis of recessivity. They pointed out the need to consider the frequency of the "affected" gene in calculations of this sort [8]. This method, however, assumes that the four *HLA* haplotypes are distinguishable in every family and therefore is subject to error, particularly when (as in *JDM*) there is association between a recessive disease and *HLA* haplotypes because the parents may share haplotypes or be *HLA*-homozygous with increased frequency. Subsequently, we [9] demonstrated the effect of the bias of ascertainment on the "effective" gene frequency and stressed the need to consider the bias when analyzing genetic data of this sort: in brief, selecting for multiple-affected sibships increases the a priori chance for *JDM-P* homozygosity of the parents that may reduce the probability of *HLA*-identity for affected sib pairs. Failure to take into account both the population frequency of the susceptibility gene and its increase by the biased distributions of parental mating types in such sibships, therefore, makes linkage more difficult to demonstrate and raises the recombination fraction estimate by increasing the number of apparent *HLA/JDM-P* crossovers. These apparent crossovers among ascertained affected sib pairs have a high probability of being noncrossovers that express inheritance of a second *JDM-P* gene from one of the parents. Barbosa et al., for example, in an analysis of linkage between *HLA* and JDM in multiplex pedigrees [10, 11], obtained a maximum lod score at  $\theta = .13$ , using a recessive gene model with 50% penetrance. Although not stated

in their papers, this result further requires the assumption that all parents are heterozygous at the *JDM* locus (equivalent to assuming a very low gene frequency and unknown parental phenotypes).

In 1979, a study of all reported affected sib pairs (including our updated material [12] and unpublished data from Copenhagen) concluded that the dominant model was untenable at any gene frequency but that under the recessive model the fit was good if the frequency of the "disease" gene (mapping very close to *HLA*) was between .2 and .45 [13]. Further data gathered in an international effort under the auspices of the Eighth International Histocompatibility Testing Workshop [14] strongly support these conclusions: the lowest  $\chi^2$  for the recessive hypothesis ( $\chi^2 = 0.44$ ) is obtained at a disease gene frequency of about .3; the minimum  $\chi^2$  for the dominant hypothesis is 16.9 at a gene frequency of .042. Thus, of the two extreme modes of inheritance, the dominant one was excluded while the recessive fit the data for a high gene frequency. This conclusion, however, has also been challenged; for example, a number of observations have been taken to suggest that genetic heterogeneity (i.e., more than one type of *JDM-P* gene) persists within the group of patients diagnosed as type 1 diabetes mellitus [15–18]. Some of these observations are of a clinical, associative type; others are mathematical arguments. The most important are:

(1) The association of the HLA antigens appears to vary in patients with different patterns of immune reactivity. HLA-B15 (and probably DR4) are found with particularly high frequency in patients with antibodies to exogenous insulin [19–21], while B8 (and DR3) are associated with the presence of antiislet-cell antibody (ICA) [18, 22–24] and diverse autoimmune diseases. No arguments have been presented, however, that in any way connect the ability to make insulin antibody *as a result of treatment* with the pathogenesis of JDM. In the case of the HLA-B8 (and DR3)-ICA association seen in some, although not all, series of patients [25, 26], again the role of these antibodies in the pathogenesis of the disease is unknown [27]. They seem, furthermore, to be present in essentially all patients regardless of HLA type shortly after onset [17, 28], and the differential frequency of HLA antigens is present only when ICA is positive long after the onset [17]. No logical connection, however, has been reported between prolonged persistence of ICA and the mechanism of expression of the genetic susceptibility to JDM. Since, on the other hand, the human homologs of the murine *H-2IR* genes in all probability map in the *HLA* region, the association of specific *HLA* alleles with specific types of immune responsiveness should be expected. The association of these *HLA* alleles with the *JDM-P* gene, whether or not strictly coincidental, should also be expected to exert the corresponding *IR*-gene influence on the immune responsiveness pattern of the patients. The mere existence of this influence is, therefore, not an argument able to discriminate between the hypotheses that more than one type of genetic determinant of the susceptibility exists and that the same *JDM-P* genetic background may be present in individuals whose *IR* genomes (and, hence, immune-responsiveness phenotypes) are different.

(2) The relationship between these two haplotypes and the age at onset is, a

priori, a better argument. B15 (and/or DR4) has been shown to be increased only in patients with very early onset [17, 18] (but for negative results, see [29]). In the material submitted to the Eighth International Histocompatibility Testing Workshop [14] (hereafter designated 8th W), some differences in the HLA associations were found, depending on the age at onset of the patients. DR4 was increased in the younger age-at-onset patients while DR3 was increased in all age-at-onset groups of patients. The population and racial heterogeneity of the patients analyzed in the 8th W, coupled with different age-at-onset cutoffs for the different samples, however, did not support a conclusive report. Svejgaard et al. [14] write that since different patient samples were truncated in different ways they find it necessary that the hypothesis of HLA-related differences in age at onset be tested in a few homogeneous populations. Some evidence exists, furthermore, that DR3-positive haplotypes may also be elevated in patients with adult-onset (type 2) diabetes [30] and particularly in ICA-positive type 2 patients [22]. Thus, even if the difference in age-at-onset association with DR3 and DR4 could be confirmed as suggested by the 8th W report [14], it would be difficult to exclude the possibility that the later onset group includes individuals with a severe form of type 2 disease, "diluting" preferentially the association with DR4. This possibility is supported by the maintenance of higher levels of insulin secretion in type 1 patients with later age at onset than in those with earlier age at onset [31]. Age-at-onset variations in the frequency of B15 are, furthermore, controversial: Ludwig et al. [32] found B15 increased especially in JDM patients with *later* onset.

(3) The relative risk (RR) associated with the B8 (*DR3*)/*B15*(*DR4*) heterozygote is higher than those attending any of the other HLA phenotypes [15, 17, 18], suggesting "overdominance" or epistasis (i.e., that the B8- and B15-associated genetic liabilities are different from each other and additive). The excessive RR to these heterozygotes, however, can be shown to be a mathematical consequence of the respective gene frequencies of *B8*(*DR3*) and *B15*(*DR4*) and of the existence of positive associations between them and an (assumed) susceptibility gene [9, 33, 34]. We have previously found [34] that if positive linkage disequilibrium exists between one (recessive) disease-susceptibility gene and two *HLA* alleles, the RR *must* be highest for the eclectic *HLA* heterozygote in all but the most extreme cases. Overdominance or epistasis is, thus, not needed to explain the observed RR values, and further, for either of them to be present, a significant excess of *DR3*/*DR4* heterozygotes must be observed over the expected Hardy-Weinberg frequencies (i.e., for the patient sample in question) [9, 14, 33]. None of the series published in detail, however, displays such an excess.

(4) It has been claimed that concordance in identical twins, ascertained through JDM in at least one member of the pair, is HLA-related (see [15] for review). B8 would be increased only in concordant pairs and B15 in both types of twins. This claim is based on an incorrect assessment of the significance of the observed differences. The lack of statistical significance of this association was pointed out by Cudworth and Woodrow [29] and, again, by Cudworth [17], two of the investigators who performed the HLA typing on the twins [35]. Cudworth states: "There

is no evidence of heterogeneity in respect of B8 and B15 in concordant compared with discordant twins . . ." [17].

(5) Recently, Rotter and Hodge [36] have derived support for genetic heterogeneity in JDM from the discussion by MacDonald [37] on Caucasian gene admixture and prevalence of JDM in American black patients. This argument was offered by MacDonald in support of a dominant mode of inheritance and against recessivity and is based on the very low prevalence of JDM in West Africa. The genetic background for the disease was therefore inferred to be absent from the West African ancestors of the present black population and to have been introduced into the American black genetic pool by their Caucasian ones. Therefore, if the gene were recessive, the homozygotes (and thus, the patients) should occur with a frequency that is a function of the square of the ratio of Caucasian to West African genes in the mixed population. In fact, the prevalence is directly related to the genetic admixture. This argument depends on the premise that the West African population and its gene pool are devoid of both JDM and its susceptibility gene(s), and would therefore be invalid if, among others, either or both of the following conditions existed in West Africa: (1) very low chance of encountering the environmental trigger and (2) very low probability of surviving beyond the onset of JDM and, thus, of being correctly diagnosed. Since at least the second condition is quite possibly true, MacDonald's argument is a priori suspect. There is, furthermore, direct evidence that for the most part the *HLA* genes of black American type 1 patients are not of Caucasian origin: Rodey et al. [38] found an excess of DR3 and DR4 among these patients, but these antigens were not in the conventional "Caucasian" haplotypes. In fact, there was no excess of HLA-B8 or -B15 in that sample. While other workers have found increased frequency of B15 in black JDM patients [39, 40], the frequency of B8 and B18 has been consistently normal, which, in the presence of significantly increased DR3, is an almost definitive proof of the non-Caucasian origin of the majority of the DR3-bearing haplotypes in these patients. MacDonald's argument, therefore, cannot be used safely in support of any hypothesis unless and until it is proven that, in fact, Caucasian *HLA* genes are invariably associated with the disease in blacks.

Scrutiny of the impressive array of observations marshaled in favor of the hypothesis of genetic heterogeneity in JDM shows the arguments to be wrong, flawed, or at least controversial. The search for solid supportive evidence continues, however, and in a recent article Barbosa et al. [41] argue that pedigrees with different patterns of affected individuals (e.g., single-generational vs. multi-generational) may reflect different patterns of inheritance. Starting from the premise that families with JDM patients in consecutive generations are likely to present a dominant mode of inheritance, Barbosa et al. [41] published a linkage analysis of 28 new multiplex pedigrees with affected individuals in two consecutive generations. They found virtually no evidence of linkage between *HLA* and a dominant *JDM-P* gene (maximum lod of 0.37 at  $\theta = .29$ ). Analysis of the data assuming recessivity of *JDM-P* with 50% penetrance and a gene frequency of

about .03 also showed no significant evidence of linkage (maximum lod of 0.33 at  $\theta = .27$ ) [41]. Barbosa et al. concluded that these results were different enough from those in the single-generation multiplex families [10, 11] to indicate that the two sets are probably not expressing a single genetic mode.

We will present arguments to show that there is no significant genetic heterogeneity between the families in the three studies of Barbosa et al. [10, 11, 41] or between those families and the ones analyzed by Falk et al. [7] and Suciú-Foca et al. [12]. We will further show that if the different types of ascertainment, and the consequent distribution of expected parental genotypes are taken into account, the three sets of data support the hypothesis of *close* linkage between *HLA* and a recessive *JDM-P* gene with penetrance  $\leq .5$ , as first formally shown by Falk et al. [7]. We will then examine data from *HLA*- and *GLO*-typed families that demonstrate that the susceptibility gene (*JDM-P*) is located nearer to *HLA* than *GLO*, thus supporting the conclusions of the formal linkage analysis and providing further evidence of the close linkage between *HLA* and *JDM-P*.

#### MATERIALS, METHODS, AND RATIONALE

##### *Formal Genetic Analysis*

The families reported in Barbosa et al. [10, 11] (B-I), Barbosa et al. [41] (B-II), and Suciú-Foca et al. [12] (SF) were analyzed using a modified version of the New York Blood Center (NYBC) linkage program (NYLIP) [42] (the families analyzed in [7] are a subset of those in [12]).

The NYBC linkage program (NYLIP) uses the conventional lod-score technique of Morton [43] and is described in detail in Falk et al. [42]. The modifications to NYLIP allow for reduced penetrance at the disease locus and adjustments to the distribution of expected parental mating types based on ascertainment bias.

In general, in families in which parents are untested or have uncertain genotypes, the probable genotypes are weighted on the basis of population gene frequencies for the traits being considered and/or phenotypic information about the parents' parents. In the case of the *JDM* families analyzed here, the expected disease-gene genotypes of the parents were further modified according to the selection bias of the family material. These modifications are particularly necessary for highly deleterious traits with reduced penetrance since the parental phenotypes are poor indicators of the underlying genotypes.

There are three mating classes that may have offspring affected with a recessive disease, namely, both parents homozygous for the "disease" *D* gene, one parent homozygous and one heterozygous, or both parents heterozygous. We have shown [9] that the expected frequencies of these three classes will be  $p^2$ ,  $2pq$ , and  $q^2$ , respectively, when there is a single affected child and where  $p$  is the population frequency of the recessive *D* gene. The changes in the proportion of the mating types to be expected if there are two, three, etc., affected children can also be calculated [9]. If, for example, the population frequency of *JDM-P* is as low as .20, then over 50% of the families with two affected children should have at least one genotypically homozygous parent, whereas a conventional analysis would assume that all non-*JDM* parents were heterozygous at the *JDM* locus. The relative proportions of the mating types to be expected are, a priori, dependent upon the number of both normal and affected children in a family. In the APPENDIX we present the general formulas for the probability of each mating class for the general case of  $m$  affected and  $n$  unaffected children. These formulas are used to adjust for bias in the linkage analysis.

Reduced penetrance is included in the calculations as described by Falk et al. [7]. If, for example, the expected proportion of *JDM-P* homozygous children is  $F$ , the expected ratio of

phenotypically normal to phenotypically affected among them will be  $F(1 - x):Fx$ , where  $x$  is the penetrance of the trait.

Barbosa et al. analyzed the families in B-I and B-II with the aid of LIPED [44]. To demonstrate that LIPED and NYLIP yield comparable results, the families in B-I were first reanalyzed by NYLIP using a very low population gene frequency without adjustments to the parental mating classes. The reanalysis produced a lod score of 3.976 for 50% penetrance and 2.456 for 100% penetrance at a recombination frequency of .13 (the values published by Barbosa et al. for B-I were 3.98 and 2.47, respectively [11]). Similarly, a family-by-family comparison (results not shown) using values extrapolated from figure 1 in B-I [11] showed very close agreement. Both computer programs yield, thus, essentially identical results.

Twenty-one families are analyzed from the B-I set, and 26 from B-II. Pedigrees 0227 and 0230 in B-II are excluded from the present analysis: family 0227, because the almost negligible amount of information would be obtained from a very young nonaffected individual, and family 0230, because the only informative parent might be homozygous for *HLA*. All of the 62 families reported by Suciú-Foca et al. [12] are included. Analysis of linkage to *HLA* is done with NYLIP, assuming a population gene frequency of .2 for the susceptibility gene, recessive inheritance with 50% penetrance, and the correction for parental mating-type frequencies. The choice of .2 for the disease gene frequency is based on the results of the 8th W [14], this value being its probable lower bound assuming recessivity. The choice of 50% for the penetrance is based on the published British twin data and is, similarly, a probable *higher* bound.

#### *The Location of the JDM-Susceptibility Gene (JDM-P) in the HLA Region*

The relative positions of *HLA*, *GLO*, and *JDM-P* may be investigated by following the segregation of *HLA* and *GLO* in doubly-informative families with two affected sibs *and* in one of whom a crossover has separated these markers. In families of this type, the sib pair should be most often *HLA*-identical and *GLO*-different, or *GLO*-identical and *HLA*-different, depending on whether the susceptibility locus lies toward *HLA* or *GLO*, respectively, with regard to the crossover site.

The families used for this analysis have been either reported partially (the *HLA* types of SF families are given in Suciú-Foca et al. [12]) or are "new." *HLA* studies for the previously reported pedigrees were done with the NIH technique; the new pedigrees were *HLA*-typed with the contrast fluorescence test [45] (a modification of the microcytotoxicity test), using 180 mono- or oligo-specific reagents. *GLO* was tested with a modification of Kömpf et al.'s technique [46]. Briefly, agarose 1% in veronal buffer (0.05 M, pH 8.6) was substituted for the starch support medium. Electrophoresis of hemolysates was done using a 3× concentrated bridge buffer at 12.5 V/cm in special trays cooled by circulating refrigerated water.

#### RESULTS AND DISCUSSION

The results of the formal linkage analysis are summarized in table 1. If all results are summed, the maximum-likelihood estimate of  $\theta$  is  $.05 \pm .03$ , with a highly significant total lod score of 7.40. This value is compatible with our observation (to be discussed below) that the recessive *JDM-P* is not farther away from *HLA* than the *GLO* locus, that is, not farther than  $\theta \cong .05$  to  $.10$  ([47] and Rubinstein et al., unpublished observation, 1981).

We have considered a single mode of inheritance for the analysis of all families. We reject the a priori assumption of Barbosa et al. that the families in B-II have distinct genetics because, even if the *JDM-P* gene is recessive, there is no choice but that there be a certain proportion of families in which both a parent and children

TABLE 1  
RESULTS OF FORMAL LINKAGE ANALYSIS OF 109 JDM FAMILIES

SOURCE	RECOMBINATION FREQUENCY								$\hat{\theta}$
	.02	.04	.06	.08	.10	.20	.30	.40	
B-I [10, 11].....	3.85	3.75	3.63	3.47	3.30	2.25	1.16	0.32	0.0
B-II [41].....	-1.78	-1.34	-1.02	-0.74	-0.53	0.04	0.12	0.05	0.30
SF [12]*.....	5.10	4.95	4.74	4.49	4.22	2.72	1.33	0.36	0.0
Total .....	7.17	7.36	7.35	7.22	6.99	5.01	2.61	0.73	0.05

\* Families 1-22, 40-46, and 51-58 of SF were analyzed in Falk et al. [7].

are affected. In fact, using the parameters mentioned above (i.e., a recessive gene frequency of .2 and 50% penetrance), we would expect 9% of all families with one affected child to have one affected parent, which is even higher than the proportion encountered by Degnbol and Green [48]. The very scant information on linkage yielded by the analysis of the B-II subset of families is also in line with this hypothesis. If the gene is recessive, and if at least one child and one parent are affected, the nonaffected parent is the potentially informative one and must have at least one *JDM-P* gene. The other gene in this parent may also be a *JDM-P* gene, in which case the family is uninformative regarding linkage. The a priori chance that this will occur is a function of the disease gene frequency and of the proportion of affected to normal among the other children as shown in the APPENDIX. In general, the higher this proportion, the higher the probability of homozygosity in the nonaffected parent and, therefore, the *lower* the probability that the family is informative.

Direct support for the rejection of genetic heterogeneity between B-II and other samples of JDM patients comes from HLA data. The frequency increase of DR3 and DR4 in B-II patients is of the same order of magnitude of that observed in other series of unrelated patients or in pedigrees with multiple cases in only one generation [12, 14].

As a separate check of the validity of the claim that the different families are, in fact, homogeneous with regard to the inheritance of JDM, we used the criterion proposed by Morton [49] to test for heterogeneity of linkage estimates in the three sets of families. Let  $z_i$  be the maximum lod score for the  $i$ th family in a sample of  $n$  families and  $Z$  be the maximum lod score for all of the families. Then, for large samples, the quantity

$$h = 4.605 \times (\sum_{i=1}^n z_i - Z)$$

is approximately distributed as  $\chi^2$  with  $n - 1$  degrees of freedom (df), if the data are homogeneous. The value of  $h$  for the material in B-I and B-II was 20.3 with 46 df ( $P > .99$ ). For all the families in the three samples,  $h$  was 42.8 with 108 df ( $P > .99$ ). Based on Morton's test, therefore, the three sets of families may, and

should be, combined. The relatively low  $\chi^2$  values coupled with the apparent differences between the results for B-II and the other data sets (by inspection) suggest a possible lack of sensitivity in the heterogeneity test for data of this sort, since the maximum-likelihood estimate of  $\theta$  for B-II alone has a large standard error (0.17). The 95% confidence interval for B-II includes  $\theta = .0$ , showing that families ascertained in this way are subject to large statistical fluctuations.

Studies of linkage in the presence of population association should be interpreted with caution, as pointed out by Spence and Hodge [50] in their demonstration that epistatic association of unlinked loci could mimic linkage. To minimize this possibility, we looked at the subset of families in which only non-JDM-associated HLA antigens segregated (i.e., those families lacking B8, B15, DR3, and DR4). In those families with non-JDM-associated antigens, the maximum-likelihood estimate of  $\theta$  was .0 with  $Z = 2.22$  (table 2). The 15 families in this group account for more than 25% of the total lod score from all 109 families. Thus, the evidence for linkage remains strong even when the influence of association is removed.

The high probabilities of close linkage (table 1) are supported by direct data on *HLA/GLO* recombination. It should be emphasized that recombinants within the *HLA* region have a "normal" frequency in our sample of JDM families [12, 33]. Initial findings of increased recombination were caused by an unusual series of 10 families [4] with 37 children, six of whom were shown to have intra-*HLA* recombinants. Each of these has been confirmed by retyping, and nonpaternity was not encountered even though testing included more than 20 other genetic systems. The rate of recombination became similar to that found in nondiabetic pedigrees as the number of families increased. In our last report [33] of 78 JDM pedigrees, the rate of recombinants to informative meioses was shown to be 7/440 for the *A/B,D* interval, and 5/498 for *A,B/D*. For the *A,B,D/GLO* interval, the rate was 9/120 in the fathers and 12/105 in the mothers. Already reported information on intra-*HLA* recombination, summarized in table 3, places the *JDM-P* gene closer to the *D*-end than to the *A*-end of *HLA*. This is also the conclusion from two other families with *B/D* recombinant diabetic children studied by Bertrams et al. [51]. In both families, an additional diabetic child was *D,DR*-identical to the recombinant. Thus, given the known distance between *GLO* and *HLA-D* ( $.05 < \theta < .1$ ) [47], recombinations in the *HLA/GLO* interval should produce independent evi-

TABLE 2  
LINKAGE ANALYSIS OF 15 FAMILIES LACKING JDM-ASSOCIATED HLA ANTIGENS

SOURCE	RECOMBINATION FREQUENCY						$\hat{\theta}$
	.00	.02	.04	.06	.08	.10	
B-I .....	1.51	1.45	1.39	1.32	1.25	1.17	0.0
B-II .....	-0.11	-0.02	0.05	0.10	0.13	0.15	0.15
SF .....	0.81	0.76	0.71	0.66	0.60	0.55	0.0
Total .....	2.21	2.19	2.15	2.08	1.98	1.87	0.0

NOTE: Families included—B-I: DD, JW, JR, DS; B-II: 216, 220, 228, 222, 224, 225; SF: 11, 37, 42, 49, 54.

TABLE 3  
INTRA-HLA RECOMBINATIONS IN AFFECTED SIB PAIRS

Family	Affected parental haplotypes (*)	Recombinant haplotype (†)	HLA segment shared by affected sibs
51. By .....	b) <u>A11, Bw35, Dw4, DR4</u> d) <u>A29, B8, Dw3, DR3</u>	c/d A28, B14 <sup>x</sup> , <u>Dw3, DR3</u>	D, DR
52. He .....	a) <u>A1, B8, Dw3, DR3</u> d) <u>A28, B27, Dw1, DR1</u>	b/a A2, Bw40 <sup>x</sup> , <u>Dw3, DR3</u>	D, DR
53. Ma .....	b) <u>A26, Bw38, Dw5, DR5</u> d) <u>A10, B8, D-, DR-</u>	c/d A3 <sup>x</sup> , <u>B8, D-, DR-</u>	B, D, DR
55. Wh .....	b) <u>A1, B8(Bw6), Dw3, DR3</u> d) <u>A28, B8(Bw6), Dw3, DR3</u>	a/b A28, B-(Bw4) <sup>x</sup> , <u>Dw3, DR3</u>	D, DR

NOTE: Data from [12], which includes complete HLA types. a, b = paternal *HLA* haplotypes; c, d = maternal *HLA* haplotypes.

\* Parental or nonrecombinant haplotypes inherited by the affected sib without intra-*HLA* recombination.

† Site of the crossover is indicated here with "x"; the segment shared is underlined.

dence either confirming the formal analysis or rejecting it. If, for instance, *JDM-P* were as far from *HLA* as the  $\theta = .13$  suggested by Barbosa et al. [11], all affected sib pairs, one of whom carries an *HLA/GLO* recombination, should be *GLO*-identical and *HLA*-half identical. Five families in our series satisfied the requirements of having two or more affected children, one of whom expressed an *HLA/GLO* crossover (table 4). (A preliminary report mentioning two families has been published [33]). In four of these families, the affected sibs shared both *HLA* haplotypes, and in the fifth, both *GLO* genes. This fifth family (family 44 (Co) [12]) would appear to separate *JDM-P* from *HLA-D*, and together with the previously discussed data, would almost inescapably place the *JDM-P* locus between *HLA* and *GLO*. This conclusion is not definite, however, since a certain proportion of the non-*JDM* parents must be *JDM-P* homozygous and able to transmit susceptibility with either allele. In the unusual family (Co), the mother (source of the *HLA/GLO* recombinant) was HLA-DR3/DR4, making this caveat even more likely since both *DR* alleles produce significant increases in the relative risks for *JDM*. These results, thus, agree with those of the formal linkage analysis in placing *JDM-P* very close to *HLA-D*.

This discussion bears on the general question of whether a single mode of inheritance underlies essentially all *JDM* cases. If only the two extreme modes are considered (i.e., dominant and recessive), dominance is excluded by the sib-pair analyses, and recessivity, while fitting those analyses, faces other problems. There is a discrepancy between the actual prevalence of *JDM* [14, 48, 52-54] and that expected from the population gene frequency of *JDM-P* (estimated from *HLA* studies in affected sib pairs) and its penetrance (derived from the concordance for monozygotic twins [6]). Numerically, given a recessive model with a population gene frequency of, say, .3, there should be a frequency of homozygous carriers of .09. If the penetrance were .5, the prevalence should be between 4% and 5%, 10

TABLE 4  
HLA/GLO RECOMBINATIONS IN AFFECTED SIB PAIRS AND TRIOS

FAMILY	HLA-GLO HAPLOTYPES IN:															
	PARENTS				PROBAND				AFFECTED SIB 2				AFFECTED SIB 3			
	A	B	D	DR	GLO	A	B	D	DR	GLO	A	B	D	DR	GLO	UNAF- FECTED SIBS
44. Co*	a)†	2	7	2	2	1	...	...	...	...	...	...	...	...	...	a c = 1
b)†	3	35	SF <sub>1</sub>	4	1	b)	3	35	SF <sub>1</sub>	4	1	...	...	...	...	a d = 2
c)†	1	8	3	3	1	c)	1	8	3	3	...	...	...	...	...	b c = 1
d)†	1	7	SF <sub>1</sub>	4	2	†2	d)	1	7	SF <sub>1</sub>	4	2	...	...	...	b d = 1
46. Gn*	a)	1	8	3	3	2	a)	1	8	3	3	2	...	...	...	b c = 2
b)	2	7	2	2	1	...	...	...	...	...	...	...	...	...	...	...
c)	1	8	3	3	1	c)	1	8	3	3	1	...	...	...	...	...
d)	11	51	SF <sub>1</sub>	4	2	†2	...	...	...	...	...	...	...	...	...	...
51. By*	a)	2	40	6	1	...	...	...	...	...	...	...	...	...	...	a c = 1
b)	11	5	4	4	2	b)	11	5	4	4	2	...	...	...	...	a d = 1
c)	28	14	-	-	1	...	...	...	...	†1	b)	11	5	4	4	2
d)	29	8	3	3	2	d)	29	8	3	3	2	...	...	...	...	b c = 5
82. We	a)	3	14	1	2	...	...	...	...	...	...	...	...	...	...	a c = 2
b)	24	51	4	2	2	b)	24	51	4	2	a)	3	14	1	2	...
c)	3	8	3	2	2	c)	3	8	3	2	...	...	...	...	...	...
d)	2	44	6	1	1	...	...	...	...	†1	...	...	...	...	...	...
115. Ma	a)	25	18	4	2	a)	25	18	4	2	a)	25	18	4	2	None
b)	3	7	2	2	...	...	...	...	...	...	...	...	...	...	...	...
c)	1	8	3	2	c)	1	8	3	2	c)	1	8	3	2	2	...
d)	9	12	8	1	†1	...	...	...	...	...	...	...	...	...	...	...

\* HLA phenotypes reported in [11]; W's are omitted to improve clarity.

† a, b = paternal *HLA* haplotypes; c, d = maternal *HLA* haplotypes.

‡ Site of recombination.

times higher than the observed figures [14, 48, 52-54]. This difference has led to the suggestion of an intermediate model according to which the penetrance for *JDM-P* heterozygous carriers would be higher than zero, although lower than for the homozygotes [14, 54]. The estimation of "expected" prevalence is, however, somewhat arbitrary. If the penetrance is taken to be .18 (e.g., the concordance found for the American monozygotic twins of patients [55]) and the gene frequency is taken to be  $\sim .2$  (i.e., about the lower value compatible with the observed data in affected sib pairs), the expected prevalence becomes  $(.2)^2 \times .18$  or .0072. For the "most likely" [14] gene frequency of .3, the expected figure would be .016. These estimates are only two- to fourfold the observed figures (*loc cit*). If it is taken into account that environmental factors play a major role in the penetrance of the disease, a two- to fourfold increase in the penetrance for the homozygous siblings of patients as compared with the homozygotes in the general population may not be unreasonable. Consider, furthermore, the difference in the rate of concordance for the British and American monozygotic twins (50% [6] vs. 18% [55]): it is obvious that these rates and, consequently, current estimations of the penetrance are subject to severe quantitative variations.

There are, in addition, inaccuracies in the *JDM-P* gene frequency estimates most often derived by the method of Thomson and Bodmer [8] from the proportions of HLA-identical and half-identical affected sib pairs [12, 14] ignoring their nonaffected sibs. In table 1A (see APPENDIX), the distribution of parental genotypes is given as a function of population gene frequency, penetrance, and sibship size and makeup. The figures can be used to estimate the change in the probability of HLA identity and half-identity for intentionally ascertained affected sib pairs as a function of the same parameters. Figure 1 shows these changes graphically for sibships with two affected siblings but of varying sizes. Large changes in gene frequency and penetrance may bring about smaller changes in the probability of HLA identi-

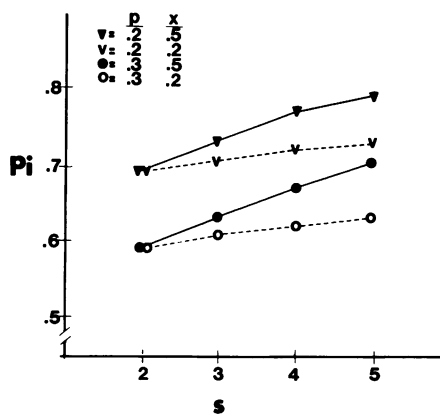


FIG. 1.—Effect of sibship size (*S*) on a priori probability of HLA-identity for an affected sib pair (*Pi*). *p* = population frequency of *JDM-P*; *x* = penetrance of *JDM-P* in homozygous state.

ty (e.g., a 50% increase in the gene frequency causes the probability to increase by only 17%). The total size of the sibship, previously not considered in these calculations, is seen to have a definite influence on this probability; an increase of sibship size from two to five brings about a change in the prior probability equal in magnitude to that caused by an increase in gene frequency from .2 to .3. Consideration of normal as well as affected sibs yields results contrary to the assumption that by studying affected sib pairs, one effectively leaves population gene frequency as the sole remaining variable.

All of these numerical uncertainties in estimates of parameters and probabilities weaken the arguments supporting the need for an intermediate model of inheritance of JDM. Those arguments [14, 54] stemmed from the apparently unresolvable discrepancies between estimates from family data and those from population data and therefore seemed to require an alternative to simple recessive or dominant models. The intermediate model, however, should be (and has been) numerically so constructed as to fit the known population prevalence and family data. As a consequence of this construction, the expected reconciliation of the discrepancies cited does not validate the model. On the other hand, its logical corollary, the postulation of different *JDM-P* genotypes for "familial" (as opposed to "sporadic") cases might be used for that purpose because of the linkage disequilibrium between *JDM-P* and *HLA-DR* alleles. In Spielman's model, for example, only about 13% of all patients in a random sample should be *JDM-P* homozygotes [54]. It should thus be expected that among these patients the frequencies of the *JDM-P*-associated *DR* alleles *DR3* and *DR4* ought to be lower than among the selected sample of patients from multiplex pedigrees among whom *JDM-P* homozygotes should comprise at least 47%. The observed allele frequencies in the two groups are not significantly different in either our material ([12] and Rubinstein et al., in preparation) or in that reported to the 8th W [14]. The "intermediate" hypothesis is, therefore, not supported by observable differences in the *HLA* allele frequencies, and its appeal is thereby reduced.

In conclusion, the formal genetic analysis of the data currently available supports the proposal [5, 6] that susceptibility to JDM is controlled by a recessive gene, with incomplete penetrance, closely linked to *HLA-DR*. It also refutes the claim [41] that more than one genetic model is needed to explain the JDM data and demonstrates that the "heterogeneities" observed in differently ascertained samples are indeed expected under the model originally proposed by us. More complicated genetic models, including intermediate inheritance, do not now appear to either be required by and analytically more consistent with the data or to involve a smaller number of assumptions.

NOTE ADDED IN PROOF: Recent papers by M. Curie-Cohen (*Tissue Antigens* 17:136-148, 1981) and B. K. Suarez and P. van Eerdewegh (*Diabetologia* 20:524-529, 1981) have independently addressed the heterogeneity question. Using several arguments, some similar to ours, both conclude that the evidence now available does not support the hypothesis of genetic heterogeneity in type 1 diabetes.

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## APPENDIX

CONDITIONAL PROBABILITIES OF PARENTAL MATING CLASSES  
BASED ON GENOTYPES OF CHILDREN

Consider a genetic disease locus with two alleles,  $G$  and  $g$ , where  $g$  is recessive to  $G$  and where the penetrance of the recessive homozygote is  $x < 1$ . Assume that a sample of families has been ascertained with one or more affected children (i.e., at least one child with the  $gg$  genotype that has expressed the disease). Parental mating pairs can then be of three types: both parents heterozygous, one parent heterozygous and one homozygous  $gg$ , or both parents homozygous  $gg$ . The relative probabilities of these three types will depend on the distribution of children in the family with respect to the trait. Because of low penetrance and strong selection against JDM individuals in the parental generation, contributions to these probabilities from phenotypically normal  $gg$  parents are minor and have been ignored.

Let  $m$  be the number of affected children in a family and  $n$  the number of normal children,  $m + n = s =$  sibship size. Using the standard definition of conditional probability:

$$P(A|B) = \frac{P(AB)}{P(B)},$$

we calculate the probability for each mating class.

*Class I:  $gg \times gg$*

$$P(I|m \text{ aff. and } n \text{ norm.}) = \frac{p^4 x^m (1 - x)^n}{\text{den}}.$$

*Class II:  $Gg \times gg$*

$$P(II|m \text{ aff. and } n \text{ norm.}) = \frac{4p^3 q \left(\frac{x}{2}\right)^m \left(1 - \frac{x}{2}\right)^n}{\text{den}}.$$

*Class III:  $Gg \times Gg$*

$$P(III|m \text{ aff. and } n \text{ norm.}) = \frac{4p^2 q^2 \left(\frac{x}{4}\right)^m \left(1 - \frac{x}{4}\right)^n}{\text{den}},$$

where den is the sum of the three numerators.

The relative frequencies are therefore:

$$\begin{array}{ccccc} \text{I} & : & \text{II} & : & \text{III} \\ p^2(1 - x)^n & : & 4pq \left(\frac{1}{2}\right)^m \left(1 - \frac{x}{2}\right)^n & : & 4q^2 \left(\frac{1}{4}\right)^m \left(1 - \frac{x}{4}\right)^n. \end{array}$$

The effect of ascertainment bias on the distribution of the expected parental mating types can be seen in table 1A.

TABLE 1A  
EXPECTED DISTRIBUTION OF PARENTAL MATING CLASSES

$p$	$x$	$s$	$m$	$n$	$gg \times gg$	$Gg \times gg$	$Gg \times Gg$
.2	.2	2	1	1 .....	.034	.310	.655
			2	0 .....	.111	.444	.444
		3	1	2 .....	.030	.301	.670
			2	1 .....	.098	.439	.463
		4	3	0 .....	.250	.500	.250
			1	3 .....	.026	.291	.684
			2	2 .....	.085	.433	.482
			3	1 .....	.225	.507	.268
		5	4	0 .....	.444	.444	.111
			1	4 .....	.022	.281	.697
			2	3 .....	.075	.425	.500
			3	2 .....	.202	.512	.285
			4	1 .....	.413	.465	.123
			5	0 .....	.640	.320	.040
		2	1	1 .....	.079	.413	.508
			2	0 .....	.213	.497	.290
		3	1	2 .....	.069	.405	.526
			2	1 .....	.191	.501	.308
		4	3	0 .....	.399	.465	.136
			1	3 .....	.060	.396	.544
			2	2 .....	.170	.503	.327
			3	1 .....	.368	.483	.149
		5	4	0 .....	.599	.350	.051
			1	4 .....	.052	.387	.561
			2	3 .....	.151	.503	.345
			3	2 .....	.338	.499	.162
			4	1 .....	.569	.373	.057
			5	0 .....	.762	.222	.016
.2	.5	2	1	1 .....	.024	.293	.683
			2	0 .....	.111	.444	.444
		3	1	2 .....	.051	.265	.721
			2	1 .....	.071	.429	.500
		4	3	0 .....	.250	.500	.250
			1	3 .....	.009	.237	.754
			2	2 .....	.045	.404	.551
			3	1 .....	.174	.522	.304
		5	4	0 .....	.444	.444	.111
			1	4 .....	.005	.211	.783
			2	3 .....	.028	.376	.597
			3	2 .....	.117	.526	.358
			4	1 .....	.340	.511	.149
			5	0 .....	.640	.320	.040
		2	1	1 .....	.057	.399	.544
			2	0 .....	.213	.497	.290
		3	1	2 .....	.035	.373	.592
			2	1 .....	.145	.509	.346
		4	3	0 .....	.399	.465	.136
			1	3 .....	.022	.343	.635
			2	2 .....	.096	.504	.400
			3	1 .....	.299	.523	.178
		5	4	0 .....	.599	.350	.051
			1	4 .....	.013	.312	.675
			2	3 .....	.062	.487	.451
			3	2 .....	.214	.562	.223
			4	1 .....	.494	.432	.074
			5	0 .....	.762	.222	.016
.3	.5	2	1	1 .....	.057	.399	.544
			2	0 .....	.213	.497	.290
		3	1	2 .....	.035	.373	.592
			2	1 .....	.145	.509	.346
		4	3	0 .....	.399	.465	.136
			1	3 .....	.022	.343	.635
			2	2 .....	.096	.504	.400
			3	1 .....	.299	.523	.178
		5	4	0 .....	.599	.350	.051
			1	4 .....	.013	.312	.675
			2	3 .....	.062	.487	.451
			3	2 .....	.214	.562	.223
			4	1 .....	.494	.432	.074
			5	0 .....	.762	.222	.016

NOTE: Frequencies of the mating classes are given for selected values of the gene frequency,  $p$ , and the penetrance,  $x$ .

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